



# Elevated Serum Free Light Chains Predict Cardiovascular Events in Type 2 Diabetes

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## OBJECTIVE

Elevated polyclonal serum immunoglobulin free light chains (FLCs; combined FLC $\kappa$ +FLC $\lambda$  [cFLC]) are associated with adverse clinical outcomes and increased mortality; we investigated cFLC and cardiovascular disease (CVD) events in type 2 diabetes.

## RESEARCH DESIGN AND METHODS

In a cohort study of 352 south Asian patients with type 2 diabetes, serum cFLC, high-sensitivity C-reactive protein (hsCRP), and standard biochemistry were measured. CVD events over 2 years were recorded and assessed using multiple logistic regression.

## RESULTS

cFLC levels were elevated significantly in 29 of 352 (8%) patients with CVD events during 2 years follow-up (50.7 vs. 42.8 mg/L;  $P = 0.004$ ). In multivariate analysis, elevated cFLC ( $>57.2$  mg/L) was associated with CVD outcomes (odds ratio 3.3 [95% CI 1.3–8.2];  $P = 0.012$ ) and remained significant after adjusting for age, albumin-to-creatinine ratio, diabetes duration, or treatment.

## CONCLUSIONS

cFLC elevation is a novel marker for CVD outcomes in type 2 diabetes that warrants further investigation.

Predicting risk of cardiovascular disease (CVD) in type 2 diabetes (1–3) using algorithms achieves only moderate performance particularly in different ethnic populations (3). An association between increased polyclonal combined immunoglobulin free light chain (cFLC, summated FLC $\kappa$ +FLC $\lambda$ ) concentrations and increased all-cause mortality has been reported (4,5); we assessed the potential of cFLC as a marker of CVD outcomes in south Asian patients with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

The United Kingdom Asian Diabetes Study was a cluster-randomized controlled trial aimed at reducing cardiovascular risk in south Asian patients with type 2 diabetes (6–8). The current study included 352 south Asian patients whose baseline fasting serum samples were available. Detailed clinical history including treatment, CVD history at baseline and over 2 years of follow-up, plus serum biochemistry at baseline (HbA<sub>1c</sub>, [img alt="Freelite logo" data-bbox="165 915 200 940"/>, and albumin-to-creatinine ratio [ACR]) were obtained. Serum cFLC (Freelite, [img alt="Freelite logo" data-bbox="165 915 200 940"/>, Binding Site Group Ltd, Birmingham, U.K.) and hsCRP (Siemens, München, Germany) levels were measured.

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Baseline characteristics of patients with and without a nonfatal or fatal CVD event over 2 years of follow-up were compared using the *t* test, Mann–Whitney *U* test for nonnormally distributed variables, and  $\chi^2$  test for categorical variables as appropriate. Factors with a *P* value <0.1 were selected for inclusion in multivariate modeling using forward stepwise logistic regression analysis to identify significant predictors of CVD events, estimating odds ratio (OR) and 95% CIs. Intervention thresholds defined in other populations may not be appropriate for south Asian patients with type 2 diabetes (7,9), so receiver operator characteristic (ROC) analysis was used to determine the optimal prognostic value of individual risk factors for nonfatal or fatal CVD events. A simple risk score was generated by summing numbers of elevated risk factors for each patient. Age, ACR (>2.5 mg/mmol in men and >3.5 mg/mmol in women), diabetes duration, treatment with insulin, statins, or ACE inhibitors were included individually as confounding factors. Statistical analysis was performed using SPSS v19 (IBM, Armonk, NY).

## RESULTS

Over 2 years, there were 7 fatal (myocardial infarction, 2; heart failure, 2; stroke, 2; and cardiac arrest, 1) and 23 nonfatal CVD events (angina, 10; myocardial infarction, 4; heart failure, 3; stroke, 2; and coronary artery bypass graft, 4). CVD events were associated with increased cFLC concentrations

(50.7 vs. 42.8 mg/L; *P* = 0.004), HbA<sub>1c</sub> (9.2 vs. 8.0% [77 vs. 64 mmol/mol]; *P* = 0.035), ACR (4.5 vs. 0.8 mg/mmol; *P* = 0.034), and age (64 vs. 55 years; *P* = 0.007), but not hsCRP (4.5 vs. 3.6 mg/L; *P* = 0.389). Patients with CVD events had longer duration of diabetes (10 vs. 6 years; *P* = 0.011) and were more likely to have previous history of CVD (34.5 vs. 15.1%; *P* = 0.008), treatment with insulin (41.4 vs. 21.4%; *P* = 0.014), statins (72.4 vs. 43.7%; *P* = 0.003), and ACE inhibitors (58.6 vs. 30.0%; *P* = 0.002).

The optimal concentrations for predicting CVD events in this population (determined by ROC analysis) were cFLC >57.2 mg/L, triglycerides >6.7 mmol/L, HbA<sub>1c</sub> >9.2% [77 mmol/mol], and systolic blood pressure (SBP) >155 mmHg. In multivariate logistic regression analysis using these values and previous history of CVD, elevated cFLC (OR 3.25; *P* = 0.012), triglycerides (OR 18.30; *P* < 0.001), and SBP (OR 3.62; *P* = 0.004) were independently associated with CVD events (Table 1, Model 1). cFLC, triglycerides, and SBP remained significantly associated with CVD risk after individually adjusting for age (Table 1, Model 2), abnormal ACR (Table 1, Model 2), and treatment with ACE inhibitors (Table 1, Model 3) or statins (Table 1, Model 4).

A risk score based on cFLC, triglycerides, and SBP identified patients with 0 (reference), 1 OR = 5.7 (95% CI 2.1–15.0; *P* = 0.001), or 2 OR = 15.4 (4.8–49.3; *P* < 0.001) risk factors; no patients were abnormal for all three risk factors. A total of 23 (79%) patients with CVD

events were abnormal for at least one risk factor. Individually, 13 (45%) patients with CVD events had elevated cFLC, 12 (41%) had abnormal SBP, and 6 (21%) had abnormal triglyceride concentrations.

## CONCLUSIONS

To our knowledge, this is the first report of a specific association between cFLC and increased risk of CVD events in type 2 diabetes. Our results are consistent with recent reports showing that high cFLC levels were prognostic for all-cause mortality where circulatory disease was a predominant cause of death (4,5). A number of factors are traditionally associated with CVD risk in western European populations (1–3,7). In our study, HbA<sub>1c</sub>, SBP, triglycerides, and CVD history were all associated with poor outcomes; however, only cFLC, SBP, and triglycerides were independently associated with increased risk of CVD events. An association between elevated hsCRP levels and increased cardiovascular risk has been reported in European populations (1,3). Although both cFLC and hsCRP are elevated in south Asians with type 2 diabetes (8,9), in this study, we found that cFLC was a better predictor of CVD risk than hsCRP. Healthy south Asians have higher hsCRP levels than white Europeans (9), so the lack of association observed in our study may simply reflect disease-specific and ethnic variations and support the need for more ethnic-specific studies.

In our study, the association between cFLC and CVD outcome was unaffected

**Table 1—Multivariate logistic regression analysis using categorical variables**

Variable	Model 1	Model 2	Model 3	Model 4
cFLC >57 mg/L	3.25 (1.29–8.19)*	3.18 (1.26–8.02)*	3.23 (1.29–8.19)*	2.64 (1.02–6.86)*
Triglycerides >6.7 mmol/L	18.30 (5.11–65.53)†	17.95 (5.01–64.29)†	18.30 (5.11–65.53)†	15.46 (4.15–57.63)†
SBP >155 mmHg	3.62 (1.50–8.74)†	3.64 (1.51–8.81)†	3.62 (1.50–8.74)†	3.43 (1.39–8.47)†
HbA <sub>1c</sub> >9.2% [77 mmol/mol]	NS	NS	NS	NS
Prior history of CVD	NS	NS	NS	NS
Age >62 years	—	NS	—	—
Abnormal ACR	—	NS	—	—
ACE inhibitor treatment	—	—	NS	—
Statin treatment	—	—	—	2.63 (1.02–6.77)*

Optimal cutoff values for cFLC, triglycerides, HbA<sub>1c</sub>, and SBP for predicting nonfatal or fatal CVD events in south Asian patients with type 2 diabetes were determined using ROC analysis and included with CVD history in a forward stepwise logistic regression model (Model 1). ORs (95% CI) are shown for each factor. NS indicates factors included in each model not associated with CVD events. — indicates factors not included in each model. Model 1 was then adjusted in separate analyses for both age and abnormal ACR (Model 2), for ACE inhibitor therapy (Model 3), and for statin therapy (Model 4). The ORs of models adjusted for insulin treatment or diabetes duration were the same as Model 1 (not shown). Factors that were significantly associated with adverse CVD outcomes are indicated. \**P* < 0.05. †*P* < 0.01. ‡*P* < 0.001.

after adjusting for age or for diabetes duration. However, diabetes occurs at a younger age in south Asian populations (7), and age at diagnosis may be a more appropriate risk marker than current age (2). Furthermore, cFLC remained independently associated with CVD risk after adjustment for insulin, statins, or ACE inhibitors. Although these treatments have been successful in reducing the risk of CVD in diabetes, the residual risk remained significant and may result from elevated triglyceride levels and chronic inflammation, which have been proposed as additional therapeutic targets (10,11). In this study, a simple risk model including cFLC, SBP, and triglycerides identified the majority of patients suffering CVD events and could therefore be useful in identifying high-risk patients that may benefit from such novel treatments. Microalbuminuria is an important risk factor for CVD (12). In this study, an abnormal ACR was not independently associated with CVD outcome and adjusting for abnormal ACR did not alter the association of cFLC with CVD risk. The lack of association between ACR and CVD may simply reflect the aggressive risk-factor management in those with microalbuminuria but needs further verification.

Serum cFLC levels represent the balance between B-cell lineage production and clearance/metabolism that occurs principally in the kidneys (13,14). cFLC concentrations are raised by chronic inflammation or renal insufficiency (13,14), which are both features of type 2 diabetes. cFLC levels also correlate strongly with the severity of renal impairment (15). Previous work from our group demonstrated that cFLC concentrations are elevated in patients with diabetes compared with healthy control subjects and were seen before overt signs of renal disease (8). Increasing cFLC concentrations may therefore provide an early indication of CVD risk in patients with diabetes (12).

While our study is limited by its size, follow-up time, and few events, the consistent association of cFLC with adverse CVD outcomes suggests that cFLC could be an important marker in the estimation of CVD risk. Further studies

involving larger cohorts are needed to examine the validity of these findings in other ethnic groups and to determine whether cFLC measurement would improve existing risk models, such as QRISK2 or the United Kingdom Prospective Diabetes Study (2). Further investigations are also required to examine the influence of factors such as obesity, hypertension, and diabetes treatments on cFLC concentrations. cFLC elevation may aid in the identification of additional therapeutic targets in patients with high residual risk.

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**Author Contributions.** All authors contributed to the study design, writing the paper, data interpretation, and had full access to all data in the study. S.B. had the original idea for the study, was responsible for data collection and analysis, and undertook the detailed statistical analysis. J.M.F. and N.T.R. were responsible for data collection and analysis and undertook the detailed statistical analysis. L.K.A. was responsible for data collection and analysis. C.A.H., S.J.H., and A.H.B. had the original idea for the study. **All authors contributed to writing the paper and data interpretation and had full access to all data in the study and final responsibility for the decision to submit for publication.** S.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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# AUTHOR QUERIES

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Q1: Please confirm that the name of author Srikanth Bellary appears as intended 

Q2: Original reference 15 was cited out of order and was renumbered as 12 so that it appears in numerical order in the text (the rest of the references were renumbered accordingly). Please verify change 